AIR QUALITY SCIENCES

What Industrial Hygienists Should Know About Environmental Mycology Methods

The Forty-Third Navy Occupational Health and Preventive Medicine Workshop

March 25, 2004

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BASICS

What is mold?
How does it grow?
How is it detected?
How is it fixed?

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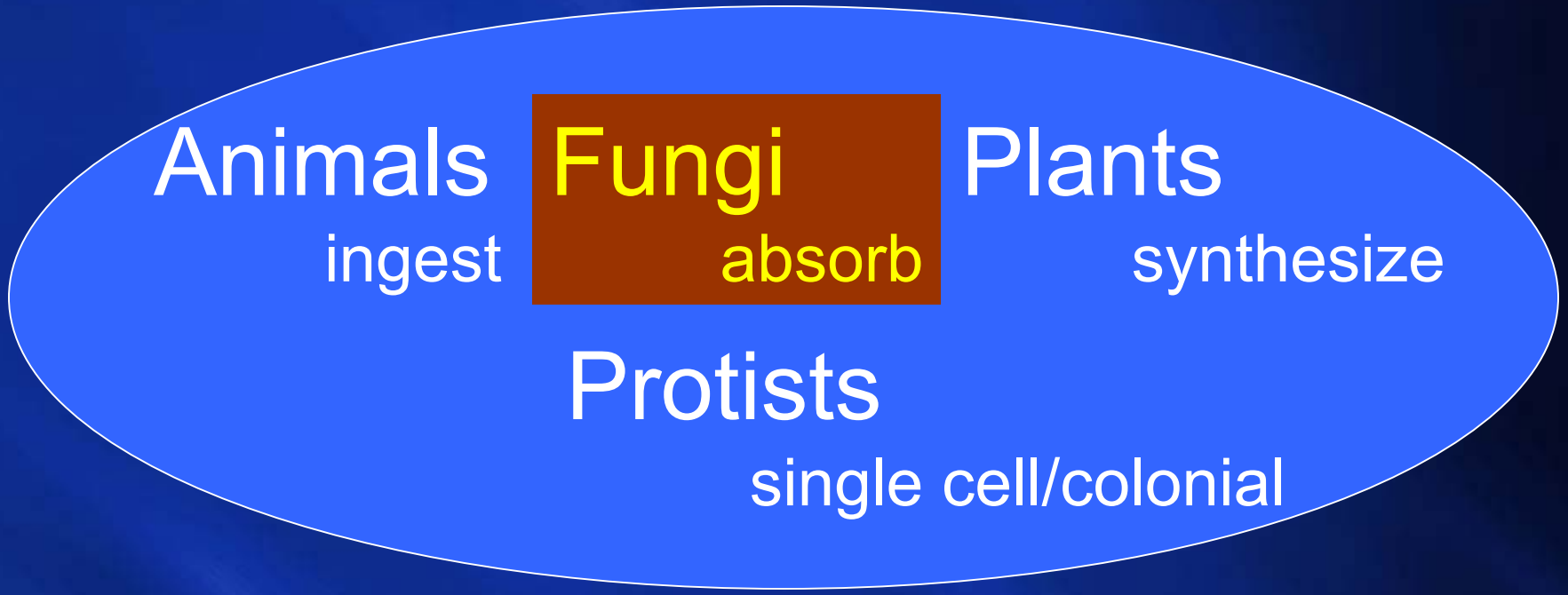
5 Kingdoms of Organisms



Bacteria

prokaryotes

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Fungi: Ecological Types

Phylloplane	Cladosporium, Epicoccum, Alternaria
Soil & litter	Penicillium, Aspergillus zygomycetes
Wood decay	Basidomycetes, (Chaetomium)

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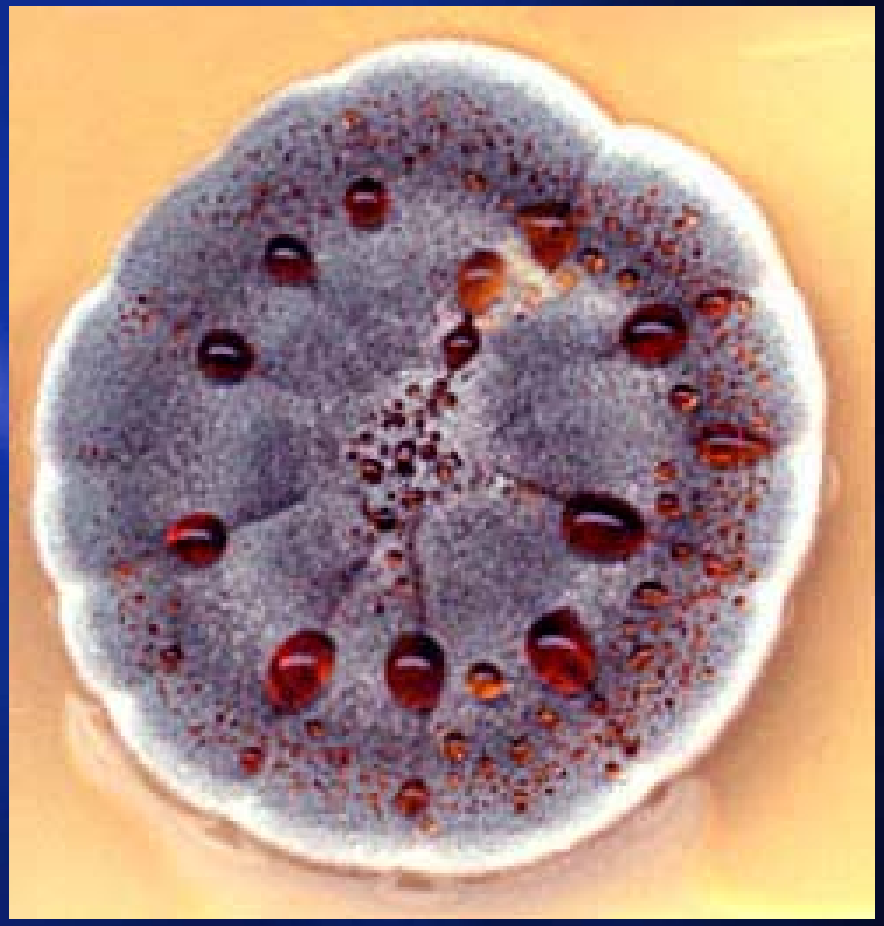
Mold: How Does it Grow?

- Terms
- Life cycles vary
- Requirements
- Optimal conditions



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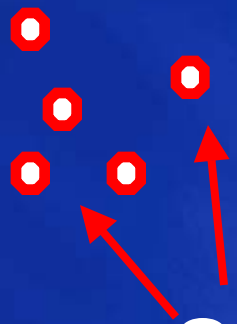
Mold Terms

Colonization vs Contamination



- Spores
- Hyphae
- Mycelium

The Mold BODY

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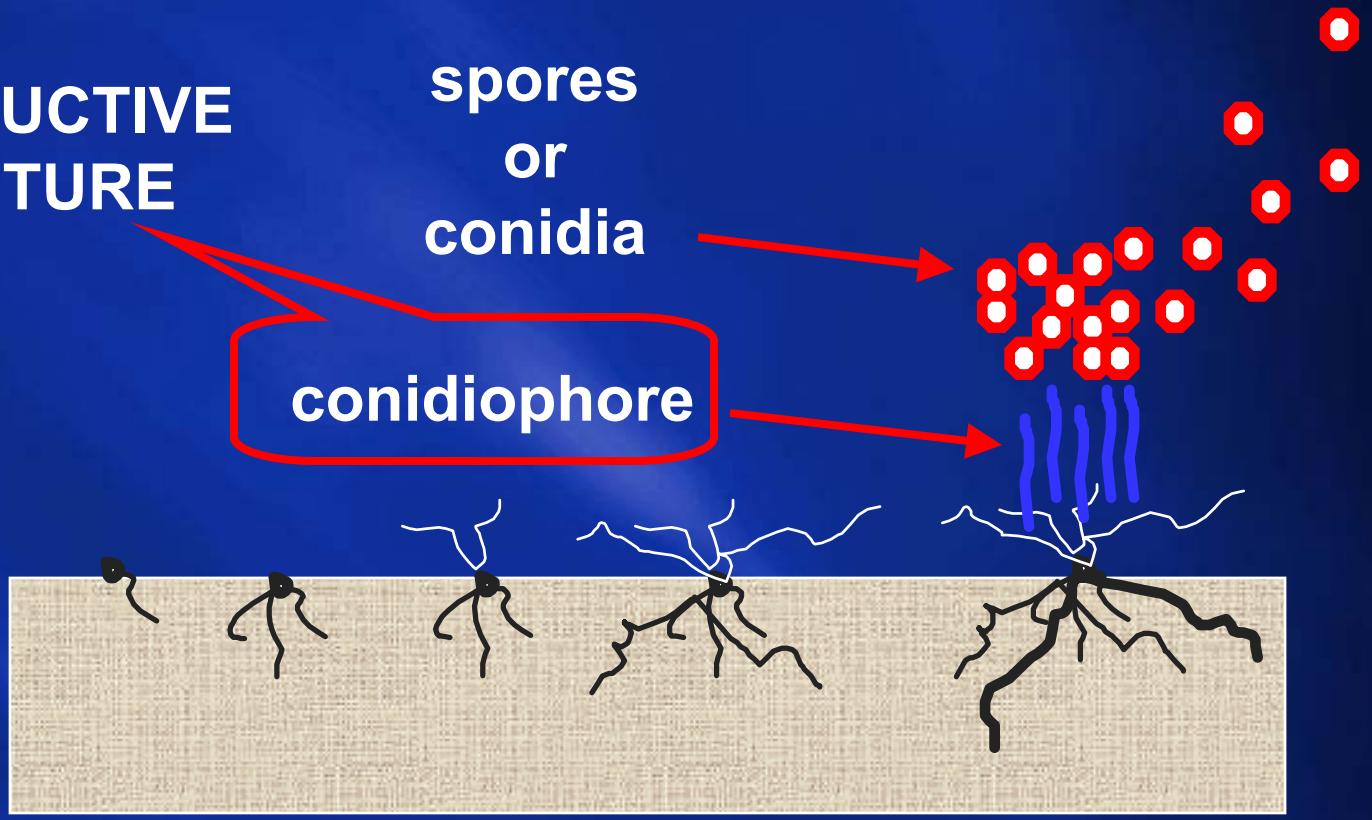
Mold Terms

REPRODUCTIVE STRUCTURE

spores or conidia

conidiophore



SOME BODY PARTS SAY MORE THAN OTHERS

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Fungi: Requirements for Growth

- **Oxygen, Temperature**
- **Food and Water**
  - **Many materials are suitable fungal food**
  - **Fungi MUST have moisture**
  - **Water is the most certain control**

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Construction Materials

c. 1270

Today….

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Fungal Food in Buildings

- Cellulose
  - Some ceiling tiles
  - Kraft paper insulation backing
  - Gyp board paper facing
- Wood
- Dirt
  - Pollen, other molds
  - Dust, insect droppings

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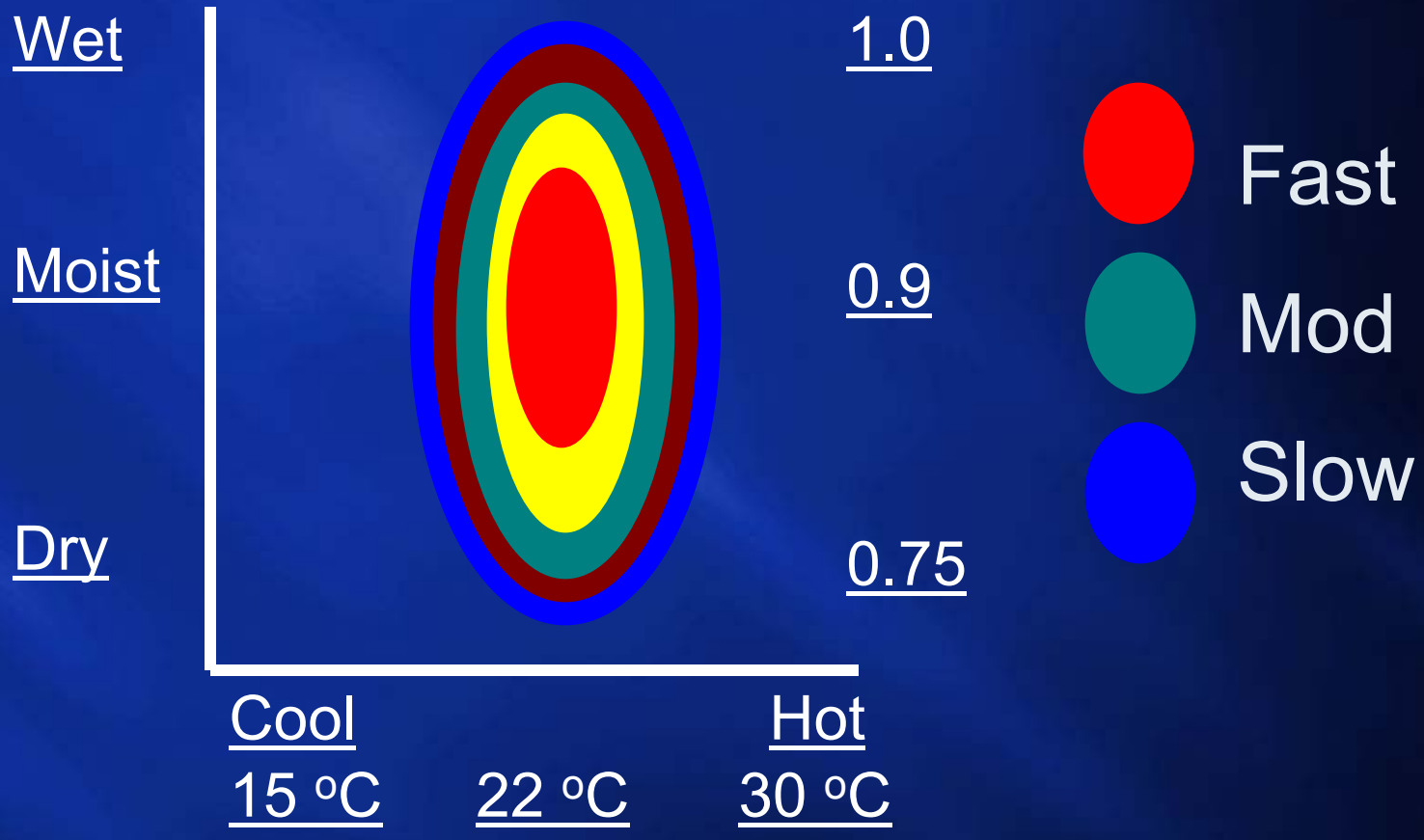
The Sweet Spot
a_w & Temperature vs. Growth Rate



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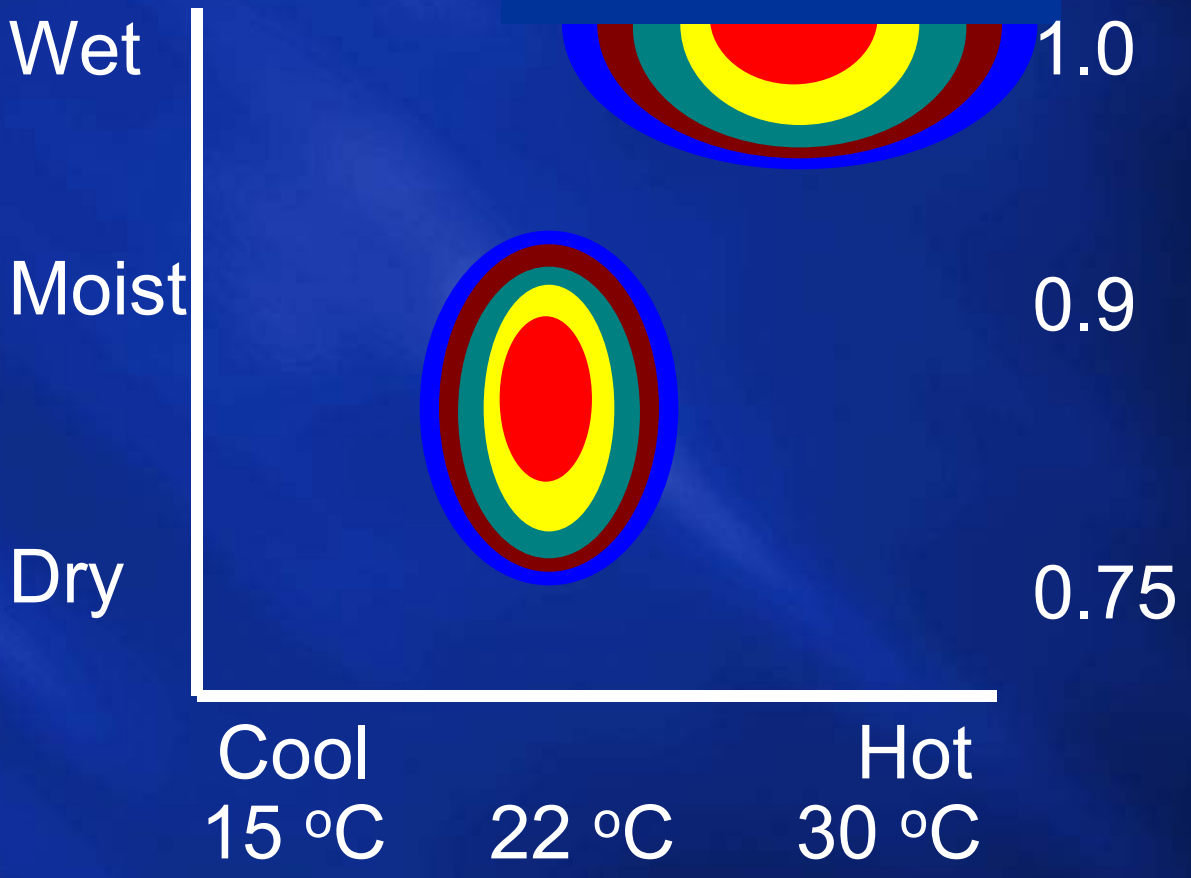
a_w & Temperature: Preferences



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Detection

Investigator: “I could throw a dart at the phone book and the house I pick would test positive for mold. And the phone book too.”

King of the Hill, “After the Mold Rush Episode”

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Detecting Fungal Growth

	Direct Microscopy	Culture-based	Metabolites
Strengths	greater breadth	greater specificity	detects disease agent
Limitations	less resolution	selectively “blind”	MVOCs DNA/PCR Immunoassay

Every method has a limitation!!

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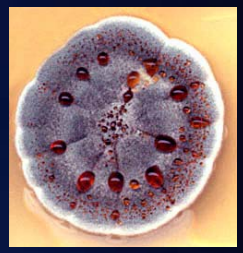

MVOCs: Microbial VOCs

- Odor "Fingerprint"
- Chemical emissions associated with microbial metabolism

  (e.g., 1-octen-3-ol, 3-methyl-1-butanol, 3-methylfuran, 2-heptanone, geosmin)
- 1st for grain, then buildings, as screening tool
- Quicker, more stable, compare types
- Target list of approximately 20 MVOCs

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MVOCs: Microbial VOCs

- Solid sorbent collection
- Calibrated sampling pump (volumetric sample)
- TAT quicker than culture
- Enables sampling of inaccessible areas
- More stable indicator of building moisture problems / mold growth

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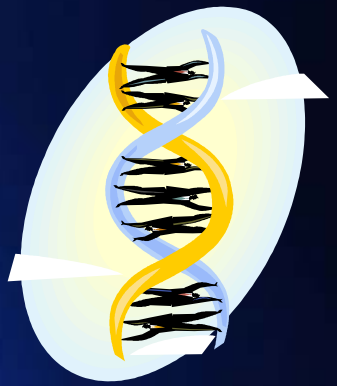
Polymerase Chain Reaction (PCR)



- Detects DNA sequences unique to mold species
- DNA molecules are duplicated exponentially in a chain reaction to detectable numbers
- Screen for presence or test for exact quantification by fluorescent labels or laser detection

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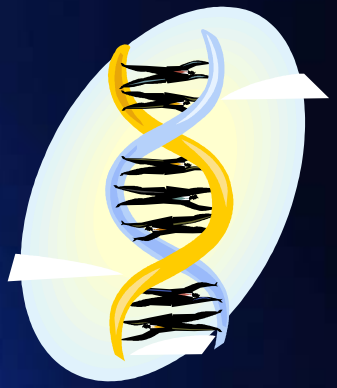
Polymerase Chain Reaction (PCR)



- Costly analytical equipment
- Delicate and complicated procedure requiring technical expertise
- 1-2 day turnaround
- $200 - 400 per sample
- Reported in units of cells/m^3
- Cell to spore ratio uncertain
- Targeted list of fungal species

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Immunoassay

- Based on antibody-antigen reaction
- Extensive clinical history (e.g. pregnancy tests)
- Qualitative and/or quantitative results

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Immediate Qualitative Results

- Antibodies specific to molds of interest pre-coated on strip surface
- Yes/No results
- Costs $10 - $20 per sample

Stachybotrys and *A. niger*

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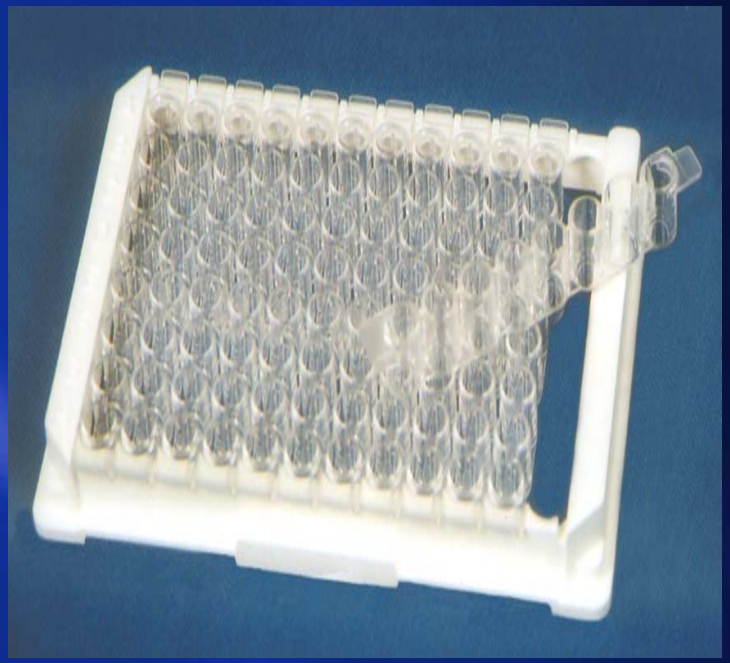
ELISA (Enzyme-Linked ImmunoSorbent Assay)

- Antibody-antigen reaction correlated to color intensity
- Mycotoxin analysis
- Spectrophotometer used to quantify
- Results in approximately 1 day
- Results expressed in ppb – ppm
- Must know type of molds for determining antibodies
- Costs $10 - $20 per sample



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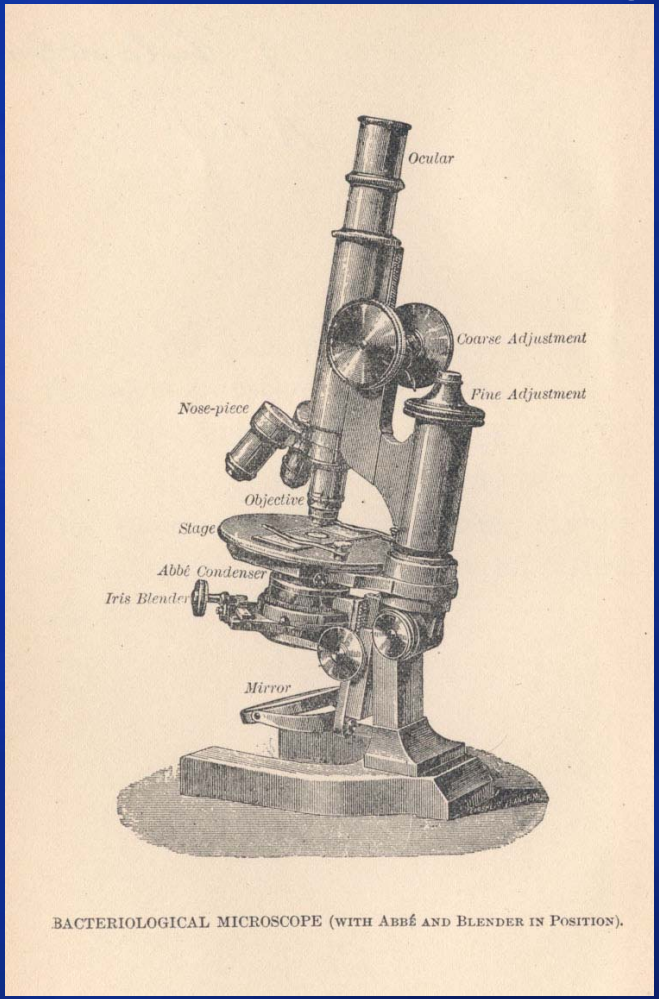
"Traditional Mycology"



BACTERIOLOGICAL MICROSCOPE (WITH ABBÉ AND BLENDER IN POSITION).

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Useful Types of Samples

- Tape slides
  - Surface
  - Non-culture
  - Growth structures
- Dust
  - Easy, Multiple
- Air culture
  - Positive identification
- Spore Trap
  - Exposure
  - Non-culture dependent
- Others:PCR,MVOC,Swab

1 method fits all?

(about as well as 1 size fits all!!)

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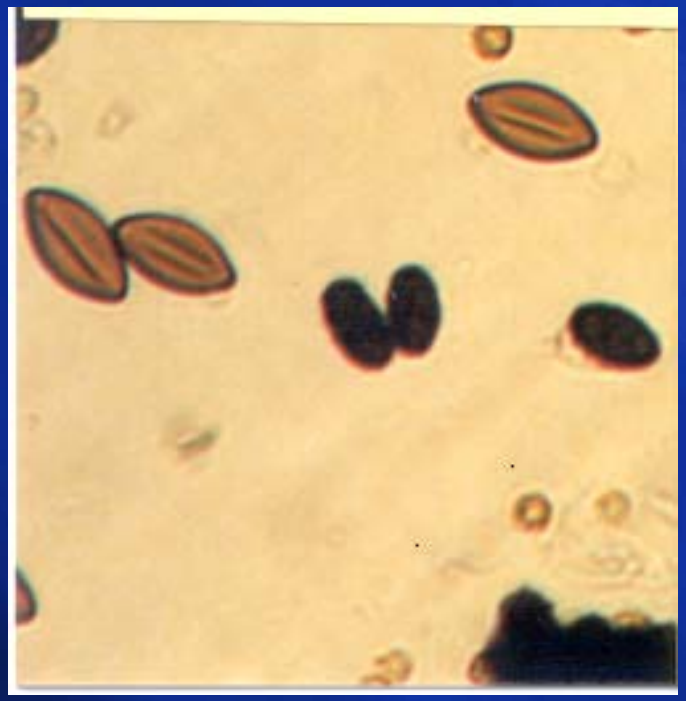
Surface Sampling: Cellotapes



- Fast, Easy
- Independent of Culturability
- Active Growth Indications
- Highly Dependent on Field Expertise
- Costs $50 - $60 per sample

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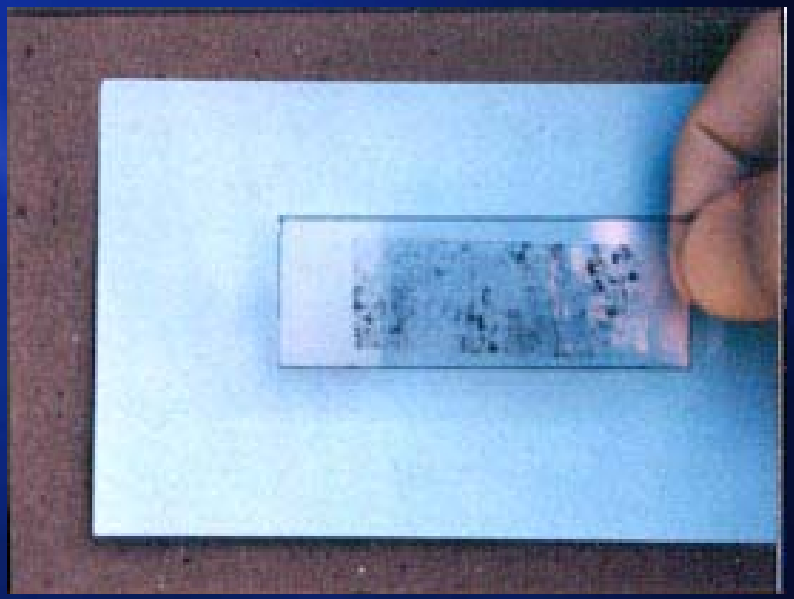
Surface Sampling: Cellotape Slides

- Use for mold on surfaces
- Lift particles with tape



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Cellotape Sample Results:

Diversity & History

Very wet

Stayed Wet

Dried slowly?

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Surface Sampling: Cellotapes

- Establishing Time of Growth
  - 1st Day – Very Good
  - 2-3 Days – Good
  - 4-5 days – Fair – Poor
  - 6 or More Days – Questionable to Dubious

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Culture Analysis

DAY 1 - Exposure / Plating

DAY 4 - Counts, Preliminary ID, Sub-cultures

DAY 7 - Further ID, Counts, Sub-cultures, Check for slow growers

DAY 10 - ID Sub-cultures, Complete ID

DAY 14 - ID Day 7 Sub-cultures

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Settled Dust

- Permits culture, antigen, chemical analysis
- Historical “picture” of building ecology
- Easy to collect
- Exposure relevance uncertain
- Costs $60 - $90 per sample for culture

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Air Culture

- Presumed route of exposure
- Variations / fluctuations
- Technically challenging
- Costs $50 - $80 per sample

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Inaccuracy of Settle Plates

Examination of Air, Soil, and Water.

Air.—Many germs are constantly found in the atmosphere about us. Bacteria unaided do not rise into the air and fly about; they usually become mixed with small particles of dirt or dust and are moved with the wind. The more dust the more bacteria, and therefore the air in summer contains a greater number than the air in winter, and all the other differences can be attributed to the greater or less quantity of dust and wind.

Methods of Examination. The simplest method is to expose a glass or dish covered with gelatine in a dust-laden atmosphere or in the place to be examined. In the course of 24 to 48 hours colonies will be seen formed wherever a germ has fallen. But this method will not give any accurate results in regard to the number of bacteria in a given space; for such a purpose somewhat more complicated methods are needed, so that a certain amount of air can come in contact with the culture media at a certain regulated rate of speed.

Ball (1900) Essentials of Bacteriology, WB Saunders

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Microbial Resistance of Building Materials

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Selecting a Laboratory

- Expertise
  - Mycologist or microbiologist
  - Environmental or clinical
- Experience, and good technical support
- Speciation
- Media, Equipment, References
- Accreditation / EMLAP

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AIHA Accreditation

- The Environmental Microbiology Proficiency Analytical Testing (EMPAT) Performance evaluation program, Lab status unknown
- The Environmental Microbiology Laboratory Accreditation Program (EMLAP), Lab status known

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Air Sampling Interpretation

- Names vs. Counts
  - Ranks
  - Indoor / outdoor ratio
- Reference to Outdoor Air Essential
- THERE ARE NO STANDARDS
- Geographical Differences

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Numbers Obscure – Taxa Reveal

CFU/m3	Taxa
40 (84.9 L)	Penicillium brevicompactum (1) Penicillium implicatum (1) non-sporulating fungi (hyaline) (1)
600 (84.9 L)	Cladosporium cladosporioides (34) Alternaria alternata (4) Aspergillus fumigatus (3) Penicillium commune (2) Rhodotorula sp. (2) Epicoccum nigrum (1) coelomycetes (1) non-sporulating fungi (hyaline) (1)

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Outdoor Bioaerosols: A Moving Target as Reference

- Season
- Diurnal Patterns
- Weather
  - Wet vs Dry
- Sampler Location
  - Height, locale, land use

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Outdoor Bioaerosols

- Source of some or all indoor spores
- Reference value
- Indoor mix reflects outdoor mix
- Indoor levels lower than out (except during snow cover)

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The Lab Report

- Should Include
  - Detection limits
  - Identifications (and amount recovered)
- Should Not Include
  - Answer “your” question
  - Provide detailed health info / easy answers
- May Include Disclaimer

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Interpreting Lab Results

- Don’t Play the Numbers - there is no “over” or “under” (no PELs)
- Balance Conclusions and Data
  - Limited Data begets Limited Conclusions
- Review Qualitative and Quantitative Results
- Use to Support Visual Inspection
- Remember the Objective

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Dust Samples: Unremarkable

Cladosporium cladosporioides (16)
non-sporulating fungi (hyaline) (7)
Epicoccum nigrum (5)
Alternaria alternata (3)
yeast (2)
Penicillium citrinum (1)

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Air Samples: Moist

Penicillium chrysogenum (65)
Aspergillus ustus (12)
Cladosporium cladosporioides (12)
Penicillium citrinum (1)
Penicillium glabrum (1)

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Tape Slides: Various

Spores	Hyphae	Fruiting Structures	Taxa
Massive	Abundant	Abundant	Stachybotrys
Abundant	None	None	Chaetomium Cladosporium Pen/Asp
Few	None	None	scattered single spores

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Remediation

- Size does matter
- Dust control
- Porous: saw
- Non porous: soap

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Objectives Of Fungal Remediation

- Physically remove colonization and mold-laden dusts
- Not to sterilize or disinfect surfaces
- Biocides (including bleach)
  - ✓Do NOT help physical removal (detergent is better)
  - ✓Do discourage infection (CDC draft guide for healthcare facilities)

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Messages

- Growth
- Sampling
- Interpretation
- Controversy
- Clean up

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Never underestimate your opponent

… We may rest assured that as green plants and animals disappear one by one from the face of the globe, some of the fungi will always be present to dispose of the last remains.

BO Dodge, Science 1939;90:379